PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 07835PC	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/IB 03/01452	International filing date *(day/month/year)* 11.04.2003	Priority date *(day/month/year)* 11.04.2003
International Patent Classification (IPC) or both national classification and IPC C12N15/62		
Applicant ESBATECH AG et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29.10.2004	08.06.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Seranski, P Telephone No. +49 89 2399-7846

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB 03/01452

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-25	as originally filed

Claims, Numbers

1-17	as originally filed
18-21	received on 11.03.2005 with letter of 09.03.2005

Drawings, Sheets

1/4-4/4	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-21
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations

   see separate sheet

The Application discloses the use of the Kluyveromyces lactis killer toxin as a selection marker in the conctruction of expression libraries in yeast. The killer toxin is comprised in a target vector and flanked by sequences encoding e.g. protein domains from e.g. single chain antibodies. By homologous recombination, the killer toxin domain is looped out of the target vector and replaced for example by a protein domain encoding the variable domain of a single chain antibody that has been prepared by randomization techniques (shuffling) beforehand. Using the killer toxin that is expressed in the target vector at a certain temperature, cells that did not recombine, will die. Vector only contamination of the randomized library is thus reduced to < 0.5% of the clones.

Prior art is restricted to the production of randomized expression libraries in yeast. US2002/0160380 for example discloses the production of combinatorial expression libraries by recombination in yeast.

US6410271, cited by the Applicant is also related to the production of expression libraries and the generation of fusion protein using homologous recombination in yeast. The methods disclosed therein are very similar to the methods of the present Application but they lack the use of any selection marker.

Also very similar to the methods used in the present Application is the disclosure of WO02/00729 that is also related to the production of single chain antibodies using randomized libraries, but also lacks the use of a selection marker.

Meinhardt et al. (1994) describe the use of the K.lactis killer toxin as a selectable marker in yeast genetics, but does not teach or suggest to use in a vector for generating libraries via homologous recombination. However, it is relevant for the use of the toxin in general.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The method of claims 1-17, the use of the K.lactis killer toxin of claims 18-19 the DNA-Vector of claim 20 and the host cell of claim 21 is novel over the prior art, thus fulfilling the requirements of Art. 33(2) PCT

The subject matter of claims 1-21 does involve an inventive step (Art.33(3) PCT) because the combination of the K.lactis killer toxin in a vector for the production of a randomized gene expression library via homologous recombination is neither taught or suggested nor clearly derivable from the disclosure of the prior art documents. As closest prior art document US6410271 is seen. The difference to the closest prior art is the use of the K.lactis killer toxin that results in the minimization of the vector background when generating the library. The technical problem to be solved can thus be formulated as to provide a method for producing a randomized gene expression library by homologous recombination with a reduced vector background. The problem has been solved in the present application by the use of the K.lactis killer toxin that is looped out of the target vector. This was neither taught nor suggested in the prior art, consequently, the subject matter of claims 1-21 and 19-20 fulfil the requirement of Art.33(3) PCT.

The subject matter of claim 1-21 is industrially applicable as set out in Art.33(4) PCT.

Clarity (Art.6 PCT)

The relative terms "at least", "preferably", "more preferably" and "in particular" used in claims 1-20 have no limiting character and leave the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claim/s unclear (Article 6 PCT).

13. The method of claims 1 to 12 wherein said target vector is introduced into said host cells in linearized form.

14. The method of claim 13 wherein said target vector is linerarized by cutting with a restriction enzyme recognizing in said first DNA sequence of said target vector said at least one unique recognition site.

15. The method of claims 1 to 14 wherein said donor sequence comprises a DNA sequence encoding a protein region, preferably a CDR region of an antibody.

16. The method of claims 1 to 15 wherein said target vector and said donor sequence are introduced into said host cells by co-transformation.

17. The method of claims 12 to 16 wherein said yeast cells are cultivated at a temperature selected from the range of 24°C to 30°C, preferably at 24°C.

18. Use of a *Kluyveromyces lactis* killer toxin as negative selection marker for the construction of randomized gene libraries and region replacement by homologous recombination.

19. Use of a *Kluyveromyces lactis* killer toxin γ-subunit as negative selection marker for the construction of randomized gene libraries and/or region replacement by homologous recombination.

20. A DNA vector which comprises the following sequences: a first target sequence for homologous recombination, a *TEF* promoter from Ashbya gossypii driving transcription of a *K. lactis* killer toxin, a DNA sequence encoding at least a γ-subunit of a *K. lactis* killer toxin and a second target sequence for homologous recombination.

21. A host cell comprising a vector of claim 20, preferably a yeast cell, more preferably a *Saccharomyces cerevisiae* cell.